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Psychophysical Evidence for a Selective Loss of M Ganglion Cells in Glaucoma

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We measured resolution acuity at 12 different retinal locations using sinusoidal gratings in a group of normals, ocular hypertensives and glaucoma patients. Resolution was measured using both stationary gratings, which selectively stimulate parvocellular ganglion cells (P cells), and gratings which phase reversed at 30 Hz, which selectively stimulate a higher proportion of magnocellular ganglion cells (M cells). With stationary gratings, peripheral resolution was found to be significantly reduced in glaucoma patients and, to a lesser extent, in ocular hypertensives. When the stimuli phase reversed at 30 Hz these differences between groups were larger. The ratio of resolution with and without phase reversal also showed a significant difference between the three groups. These results provide strong psychophysical evidence for a selective loss of M ganglion cell density over P ganglion cell density in glaucoma. © **1997 Elsevier Science Ltd. All rights reserved.**

Ganglion cells Glaucoma Magnocellular pathway Peripheral resolution

INTRODUCTION

In the fovea, the limiting factor for visual acuity is the quality of the eye's optics in that spatial frequencies higher than the sampling density of the retina do not get through (Campbell & Gubisch, 1966; Williams, 1985a,b) and the optics, in effect, act as a low-pass filter.

Evidence exists, however, that outside the fovea this is not the case. Although optical quality deteriorates peripherally (Green, 1970; Millodot *et al.*, 1975; Jennings & Charman, 1981) the density of the retinal sampling array deteriorates even faster (Perry & Cowey, 1985; Curcio & Allen, 1990), meaning that the limiting factor in peripheral vision is retinal sampling, in particular the sampling density of the coarsest array in the retinal processing sequence, the ganglion cells. Strong evidence for the sampling limited nature of peripheral resolution comes from the observations of aliasing reported in peripheral vision (Coletta & Williams, 1987; Smith & Cass, 1987; Thibos *et al.*, 1987b). Aliasing occurs when a stimulus is undersampled by the underlying sampling array and means that a grating stimulus with a mean luminance the same as its surround can be detected but not resolved.

Whereas peripheral grating detection acuity is limited by the receptive field size of retinal ganglion cells, peripheral resolution acuity is limited by ganglion cell sampling (Thibos *et al.*, 1987a). Specifically, the minimum angle of resolution (MAR) is equal to the spacing of retinal ganglion cells. Grating resolution in peripheral vision has been shown to closely match the predicted resolution, based on anatomical counts of ganglion cells in monkey (Thibos *et al.*, 1987a) and human (Anderson *et al.*, 1992; Dacey, 1993).

However, previous measurements of peripheral resolution have predominantly employed stationary sinusoidal gratings which selectively stimulate the ganglion cells which project to the parvocellular layers of the lateral geniculate nucleus. These stimuli are more sensitive to stationary stimuli (Derrington & Lennie, 1984) and begin to drop out of the resolution task when temporal frequency exceeds 10 Hz (Anderson *et al.*, 1995). A grating stimulus which phase reversed at 30 Hz would stimulate fewer P cells and more M cells, which are sensitive to higher temporal frequencies (Derrington & Lennie, 1984) and appear to be damaged earliest in glaucoma (Quigley *et al.*, 1987). Such a stimulus also yields sampling limited resolution performance in peripheral vision (Anderson, 1996) and could be used to measure resolution that is mediated by a higher proportion of M cells.

We believe that evidence for a selective loss of M to P cell density in glaucoma has hitherto been of a mainly anatomical nature. We say this because, although much physiological and psychophysical work has been conducted in an attempt to isolate M cell function in glaucoma, in order to demonstrate a *selective* loss psychophysically it is necessary to use stimuli that are designed to stimulate *both* M and P cells separately and

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compare differences in performance with both. Even from an anatomical standpoint there is some controversy as to whether there is indeed a selective loss of M ganglion cells at all (Morgan, 1994). Measurements of resolution acuity using both stationary and phase-reversing gratings designed to selectively stimulate both P and M cells respectively in normals, ocular hypertensives and glaucoma patients could yield psychophysical evidence for the existence or non-existence of a selective loss of M ganglion cell numbers in glaucoma.

METHODS

Apparatus

We devised a "resolution perimeter" which measured grating resolution at 12 locations in the visual field. The stimuli were circular patches of sinusoidal grating with the same mean luminance as the surround and were generated on a 17 inch high resolution computer monitor (Eizo) using a visual stimulus generator VSG2/3 (Cambridge Research Systems). The stimuli included four stimuli of 3 deg diameter at 10 deg eccentricity and eight stimuli of 4.5 deg diameter at 20 deg eccentricity. The fixation point was a 1 deg red cross in the centre of the screen.

Psychophysical methods

Subjects sat with their chin on a chin rest at 0.33 m from the screen while viewing the fixation cross. Only the left eyes of all subjects were tested. Subjects were optically corrected for the distance of the screen and the eye not in use was patched. Stimuli were presented randomly at each of the 12 locations using a 2AFC psychophysical procedure, where the observer had to indicate whether the orientation of the grating was horizontal or vertical by pressing one of two buttons. Stimulus duration was 0.5 sec and the subject had 5 sec to respond otherwise the test went to the next stimulus and repeated the previous stimulus at the end of the set of 12 presentations. Pressing one of the two response buttons triggered the next stimulus. All 12 locations were presented 15 times. Three correct responses caused a 10% increase in spatial frequency and one incorrect response caused a 10% decrease in spatial frequency at each location. This gave, on average, four reversals for each location. Resolution threshold for each location was calculated as the mean of the reversal values. The mean and standard deviation for resolution at all 12 locations was then calculated for each subject.

Subjects were given a short practice period lasting about 3 min to familiarize themselves with the procedure. The test was conducted twice: once using stationary gratings and once using gratings which phase-reversed sinusoidally at 30 Hz. The order of tests was randomized between subjects. Each test lasted on average 5–6 min.

Subjects

We tested three groups of subjects, recruited mainly from the glaucoma out-patient clinic in the eye hospital, all groups age matched with similar standard deviations. Subjects were catagorized by the second author (COB), who is a consultant ophthalmologist in charge of glaucoma services in the hospital.

1. Eight normals, defined as having normal optic disc appearance without pallor, intraocular pressure (IOP) of 20 mm Hg or below and no demonstrable visual field loss as measured using the Humphrey 24-2 programme. This group was recruited from catering, nursing and ancillary staff within the hospital and the spouses of attending out-patients (mean age 66.2 years, SD 6.9 years) and were not on any form of ocular medical therapy.
2. Seven ocular hypertensives, recruited from the glaucoma prevention clinic of the hospital. Of these, four were high risk, defined as having IOP greater than 25 mm Hg and optic disc cupping greater than 0.6 and no demonstrable visual field defect using Humphrey 24-2 on at least two occasions. These subjects were on therapy in the form of beta-blockers. The other three were medium risk defined as either: IOP greater than 25 mm Hg and optic disc cupping less than 0.6, or IOP 21–25 mm Hg and optic disc cupping greater than 0.6, both with no measureable visual field loss by Humphrey 24-2 on at least two occasions (mean age 68.9 years, SD 10 years). These three subjects were not on therapy at the time of the study.
3. Eight glaucoma patients, defined as having pallor and cupping of the optic discs and visual field loss, defined as three or more adjacent points at least 5 dB below normal for age, as measured using the Humphrey 24-2 programme on at least two occasions (mean age 64.7 years, SD 7.4 years). These subjects included three low tension glaucomas (IOP < 21 mm Hg) and five primary open-angle glaucomas (IOP 21–30 mm Hg) and ranged from very early glaucoma to quite advanced field loss, as measured by Humphrey perimetry. All subjects were under therapy except one who was newly diagnosed.

Subjects were determined by the ophthalmologist (COB) to have no significant other ocular pathology or a history of ocular surgery. Central visual acuity in the eye being tested was 6/9 or better with no significant lens opacity, as determined by slit-lamp biomicroscope and direct ophthalmoscope.

RESULTS

The results for the stationary grating test for all three groups are shown in Fig. 1(a). Data points represent the mean resolution across the 12 locations. Differences between groups were analysed using ANOVA to test for significance at the 5% level. Resolution was higher in the normal group than the ocular hypertensive group and higher in the ocular hypertensive group than the glaucoma group (norm 3.6 vs OHT 2.84 vs glaucoma 2.44 c/deg). These differences were significant. It can be

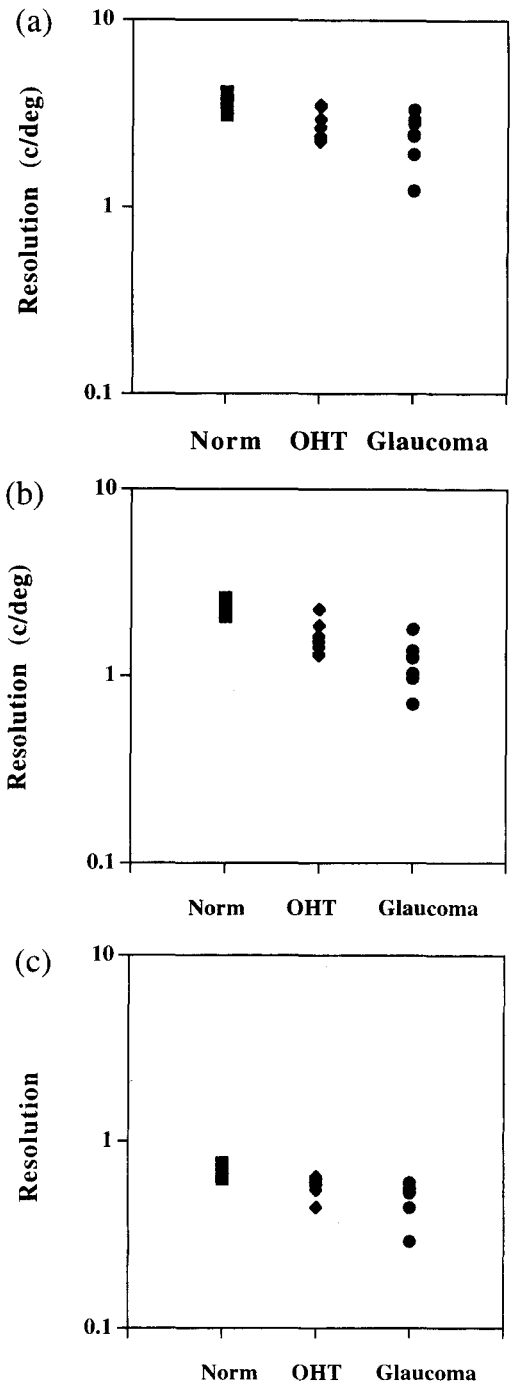

FIGURE 1. Mean resolution thresholds across all 12 retinal locations for normals, ocular hypertensives and glaucoma patients. (a) Stationary stimuli; (b) phase reversal at 30 Hz; (c) resolution ratio with/without 30 Hz phase reversal.

observed that the data points for the normals are closely clustered together (SD 10%), with greater spread for the points for ocular hypertensives (SD 16%) and glaucomas (SD 26%). There is a small overlap between normals and glaucoma patients, which is to be expected given that the range of glaucoma patients varied from very early to more advanced, and the fact that points represent an average of all field locations.

Figure 1(b) shows the values for the 30 Hz phase reversal test for all three groups. Resolution for all groups was lower than in the stationary gratings case. As before, resolution was significantly higher for normals than ocular hypertensives (2.45 vs 1.6 c/deg) and significantly higher for ocular hypertensives than glaucoma patients (1.6 vs 1.19 c/deg), but this time the differences between groups was larger than in the stationary stimulus case (ratio OHT/normal 0.65 cf. 0.79 previously; glaucoma/OHT 0.74 cf. 0.86 previously). The points for the normal subjects are again closely clustered together (SD 8%) and there is greater spread for ocular hypertensives (SD 18%) and glaucomas (SD 27%). However, in this case there is no overlap between normals and glaucoma patients.

The data in Fig. 1(c) represent the ratio of resolution with and without 30 Hz phase reversal, calculated for individual subjects. Once more, the highest values are for normals, followed by ocular hypertensives with glaucoma patients yielding the lowest ratios. The differences between groups were again significant (norm 0.683 vs OHT 0.589 vs glaucoma 0.499 c/deg). Normals are once more very closely clustered and there is no overlap between glaucoma patients and normals. These data indicate a selective loss of resolution with phase reversal, compared to resolution without phase reversal in glaucoma patients and some ocular hypertensives.

Localized losses of resolution were also apparent in all glaucoma patients. Figure 2(a) is the Humphrey visual field plot and resolution perimetry results for subject WK, who was a normal observer. Figure 2(b) is the Humphrey visual field plot and resolution perimetry measurements for subject HR who was the most advanced glaucoma patient in the study. His pattern of field loss closely matches his localized resolution deficits measured with the resolution perimeter. Figure 2(c) represents a glaucoma patient (ES) with localized nasal and paracentral defects. Again, the pattern of field loss closely resembles the resolution values at each field location and even the "normal" parts of the field show suspiciously low resolution values, particularly for the phase reversal case. Figure 2(d) shows the results for an early glaucoma patient (AH) and the nasal loss as measured by Humphrey also yields a correspondingly reduced resolution, both with and without flicker. However, as before, even the "normal" parts of the field show low resolution values compared to other normal subjects.

DISCUSSION

Peripheral resolution acuity measured using stationary sinewave gratings is significantly reduced in glaucoma patients and some ocular hypertensives, indicating that the underlying ganglion cell density is also reduced. The difference between groups is greater when the gratings phase reverse at 30 Hz and the ratio of resolution with and without phase reversal is also reduced in glaucoma patients and some ocular hypertensives. This is clear psychophysical evidence for a selective loss of flicker-sensitive ganglion cells in glaucoma. As mentioned, previous evidence for a selective loss of M cells over P

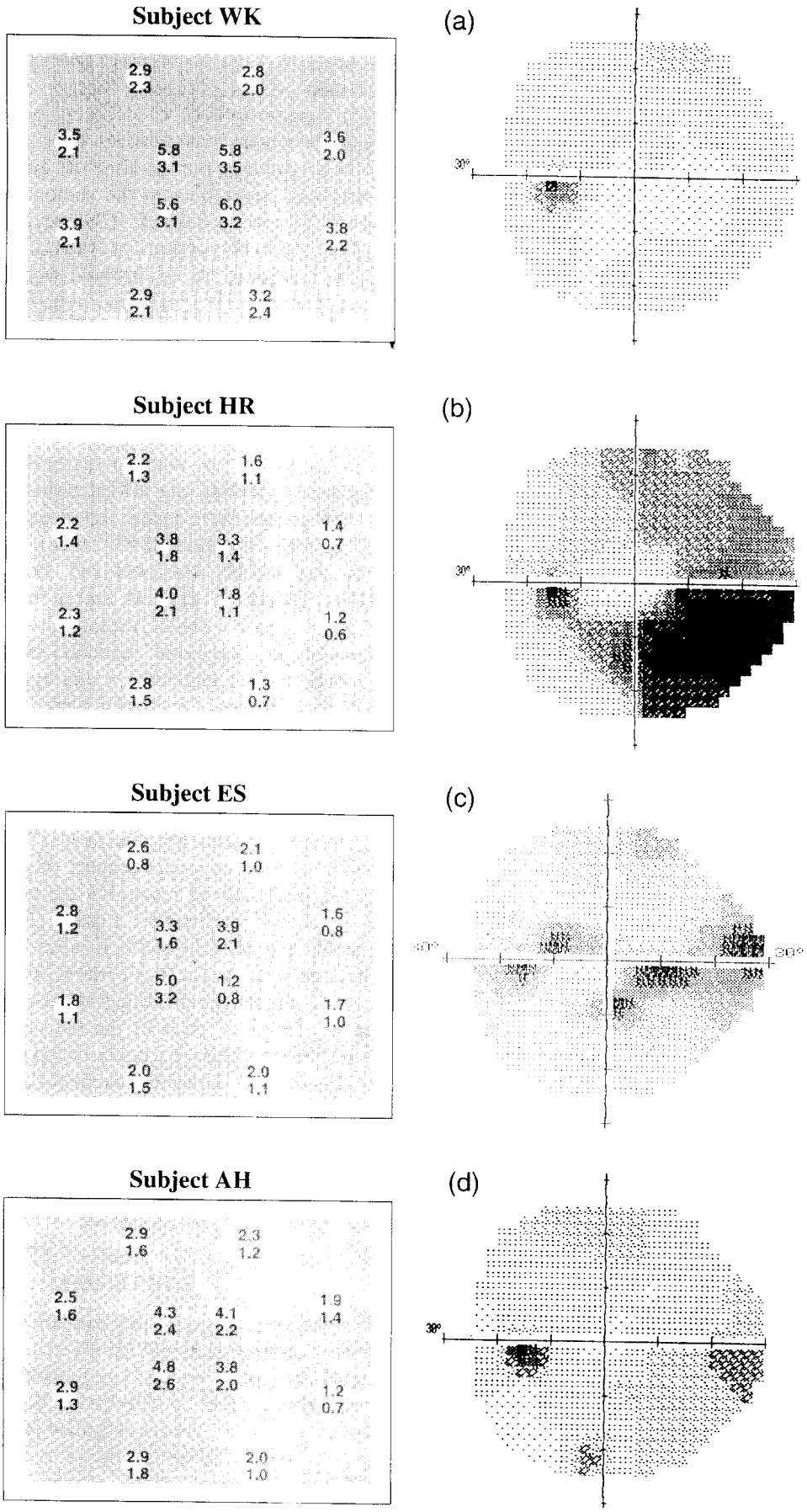

FIGURE 2. Resolution perimetry results (c/deg) and Humphrey visual field plots for four subjects, one normal (a) and three glaucoma patients (b–d). Upper value at each resolution perimetry location represents resolution without phase reversal and lower value at each location represents resolution with phase reversal.

cells has been predominantly of an anatomical nature in that psychophysical stimuli that selectively stimulated both M and P cells separately have not been employed on the same subject, allowing no direct comparison of the loss of one type of cell with another.

In general, the loss of resolution in glaucoma was greater for patients showing greater field loss as measured by Humphrey perimetry. Also, the ocular hypertensive patients displaying low resolution were usually the high risk ones. It is likely that these subjects may actually represent early glaucoma patients with field loss as yet unmeasurable by conventional perimetry.

Although significant differences exist between groups in this study, we expect the differences to be greater if the analysis were carried out in a retinally localized manner, owing to the localized nature of glaucomatous field loss. In order to do this properly, however, it would be necessary to have information on the expected normal resolution values for each field location, since different locations have different ganglion cell densities (Curcio & Allen, 1990) and yield different resolution performance in normals (Rovamo *et al.*, 1982; Anderson *et al.*, 1992). Also, ganglion cell density and, therefore, peripheral resolution, may also be reasonably expected to vary with age, meaning data on localized resolution in a range of normal patients of different ages are required. Indeed, this age variation may be different for M cells than P cells. This is a topic for immediate further research. The fact that some high risk ocular hypertensives displayed localized deficits in peripheral resolution performance, particularly with phase-reversing gratings, while simultaneously displaying normal Humphrey fields suggests that there is indeed a localized loss of ganglion cell density in these subjects and they may represent early glaucoma. Data on the expected localized resolution performance in normals of different ages would be useful in deciding whether or not these apparent losses in some subjects are significant and represent early disease.

This type of test which uses phase-reversing gratings, rather than stationary ones, to measure localized M ganglion cell density could prove useful in the detection of glaucoma at an earlier stage. In fact it may be the case that the *ratio* of resolution with/without flicker, which would indicate the presence of a selective loss of M cells, is the best indicator of the presence of glaucoma of all. Further research is also required to investigate differences in the ratio of loss in different types of glaucoma and may indicate differences in the pathogenesis of the disease in, e.g, low tension vs primary open angle glaucoma.

CONCLUSIONS

These results indicate that there is loss of peripheral resolution acuity in glaucoma and this loss is greater when measured with a 30 Hz phase reversing sinusoidal grating which stimulates a higher proportion of M ganglion cells. This is strong psychophysical evidence for a selective loss of M ganglion cells over P ganglion cells in glaucoma and could lead to a new test for the early detection of glaucoma.

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